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<b>(54) Title:</b> RECOMBINANT CYTOMEGALOVIRUS VACCINE  <b>(57) Abstract</b>  The present invention provides a non-defective adenovirus recombinant expression system for the expression of an immunogenic fragment of the HCMV gB subunit, said recombinant HCMV-expressing adenovirus being useful as a vaccine.  <div style="text-align: center; font-size: 2em; font-family: cursive;">09/ 322352</div>		

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## RECOMBINANT CYTOMEGALOVIRUS VACCINE

5 This work was performed with government support under National Institutes of Health grants AI-07278 and HD-18957. The U.S. government has certain rights in this invention.

### Field of the Invention

10 The present invention refers generally to a recombinant human cytomegalovirus vaccine, and more specifically to a subunit vaccine containing fragments of a HCMV major glycoprotein complex subunit gB gene.

### Background of the Invention

15 Cytomegalovirus (CMV) is one of a group of highly host specific herpes viruses that produce unique large cells bearing intranuclear inclusions. The envelope of the human cytomegalovirus (HCMV) is  
20 characterized by a major glycoprotein complex recently termed gB or gCI, which was previously referred to as gA. HCMV causes cytomegalic inclusion disease and has been associated with a syndrome resembling infectious mononucleosis in adults. It also induces complications  
25 in immunocompromised individuals.

CMV infection in utero is an important cause of central nervous system damage in newborns. Although the virus is widely distributed in the population, about 40% of women enter pregnancy without antibodies and thus are  
30 susceptible to infection. About 1% of these women undergo primary infection in utero. Classical cytomegalic inclusion disease is rare; however, a proportion of the infected infants, including those who were symptom-free, are subsequently found to be mentally  
35 retarded.

Preliminary estimates based on surveys of approximately 4,000 newborns from several geographical areas indicate that the virus causes significant damage of the central nervous system leading to mental  
5 deficiency in at least 10%, and perhaps as high as 25%, of infected infants. Assuming that about 1% of newborn infants per year excrete CMV and that about one fourth of those develop mental deficiency, in the United States this means approximately 10,000 brain-damaged children  
10 born per year. This is a formidable number, particularly in view of the ability of these children to survive [J. Infect. Dis., 123 (5):555 (1971)].

HCMV in humans has also been observed to cause serious complications and infections in the course of  
15 organ transplantations, especially with kidney and liver transplants.

Several HCMV vaccines have been developed or are in the process of development. Vaccines based on live attenuated strains of HCMV have been described.  
20 [See, e.g., S. A. Plotkin et al, Lancet, 1:528-30 (1984); S. A. Plotkin et al, J. Infect. Dis., 134:470-75 (1976); S. A. Plotkin et al, "Prevention of Cytomegalovirus Disease by Towne Strain Live Attenuated Vaccine", in Birth Defects, Original Article Series, 20(1):271-287  
25 (1984); J. P. Glazer et al, Ann. Intern. Med., 91:676-83 (1979); and U. S. Patent 3,959,466.] A proposed HCMV vaccine using a recombinant vaccinia virus expressing HCMV glycoprotein B has also been described. [See, e.g., Cranage, M. P. et al, EMBO J., 5:3057-3063 (1986).]  
30 However, vaccinia models for vaccine delivery are believed to cause local reactions. Additionally, vaccinia vaccines are considered possible causes of encephalitis.

Adenoviruses have been developed previously as efficient heterologous gene expression vectors. For example, an adenovirus vector has been employed to express herpes simplex virus glycoprotein gB [D. C. Johnson et al, Viol., 164:1-14 (1988)]; human immunodeficiency virus type 1 envelope protein [R. L. Dewar et al, J. Virol., 63:129-136 (1988)]; and hepatitis B surface antigen [A. R. Davis et al, Proc. Natl. Acad. Sci., U.S.A., 82:7560-7564 (1985); J. E. Morin et al, Proc. Natl. Acad. Sci., U.S.A., 84:4626-4630 (1987)]. Adenoviruses have also been found to be non-toxic as vaccine components in humans [See, e.g., E. T. Takajui et al, J. Infect. Dis., 140:48-53 (1970); P. B. Collis et al, J. Inf. Dis., 128:74-750 (1973); and R. B. Couch et al, Am. Rev. Respir. Dis., 88:394-403 (1963)].

There remains a need in the art for additional vaccines capable of preventing CMV infection by generating neutralizing antibody and cellular responses to CMV in the human immune system.

#### Summary of the Invention

In one aspect, the present invention provides a non-defective recombinant adenovirus containing a fragment of a gB subunit of the HCMV free from association with any additional human proteinaceous material. In this recombinant adenovirus, the HCMV subunit is under the control of regulatory sequences capable of expressing the HCMV gB subunit fragment *in vitro* and *in vivo*.

Another aspect of the present invention is a vaccine composition comprising a non-defective recombinant adenovirus, as described above.

In a further aspect, the invention provides a method of vaccinating a human against HCMV comprising administering to the patient the recombinant adenovirus

containing the subunit gene encoding a gB protein fragment in a vaccine composition. The inventors have found that this method of presenting these HCMV gene fragments to a vaccinate is particularly capable of eliciting an immune response.

In still a further aspect the invention provides an adenovirus-produced gB subunit fragment, which fragment may also form vaccine compositions to protect humans against HCMV. Currently, the preferred fragment comprises about amino acids 1 to about 303 of the gB protein SEQ ID NO:2, gB<sub>1-303</sub>.

Other aspects and advantages of the present invention are described further in the following detailed description of preferred embodiments of the present invention.

#### Brief Description of the Drawings

Fig. 1A illustrates diagrammatically the cloning of the gB gene into the early region 3 (E3) transcription unit of Ad5. Represented are the 3.1kb fragment containing the gB gene by the open box; the adenovirus sequences extending from 59.5 to 100 mu (except for the deletion of the 78.5 to 84.7 mu length) by the filled portion of the circle; the large BamHI fragment of the pBR322 by the thin line of the circle. In the figure, the restriction enzymes are identified as follows: X is XbaI, B is BamHI.

Fig. 1B illustrates diagrammatically the construction of the recombinant adenovirus virus Ad5/gB, containing the gB gene of the Towne strain of HCMV described in Example 1. This figure shows the 59.5 mu to 76 mu region where homologous recombination occurs (as indicated by the crossed lines) between wild type Ad5 viral sequence and the adenovirus sequences present on the pAd5 plasmid containing the gB gene. The plaque

purified recombinant virus retains the cloning XbaI sites and the direction of transcription of the gB gene from the E3 promoter is indicated by the bent arrow. Restriction enzymes are as identified above.

5

#### Detailed Description of the Invention

The present invention provides novel immunogenic components for HCMV which comprise an adenovirus expression system capable of expressing a  
10 selected HCMV subunit gene fragment *in vivo*. Alternatively the selected subunit fragment for use in an immunogenic composition, such as a vaccine, may be expressed in, and isolated from, the recombinant adenovirus expression system.

15 As provided by the present invention, any adenovirus strain capable of replicating in mammalian cells *in vitro* may be used to construct an expression vector for the selected HCMV subunit. However, a preferred expression system involves a non-defective  
20 adenovirus strain, including, but not limited to, adenovirus type 5. Alternatively, other desirable adenovirus strains may be employed which are capable of being orally administered, for use in expressing the CMV subunit *in vivo*. Such strains useful for *in vivo*  
25 production of the subunit in addition to adenovirus-5 strains include adenovirus type 4, 7, and 21 strains. [See, e.g., Takajuji et al, cited above]. Appropriate strains of adenovirus, including those identified above and those employed in the examples below are publicly  
30 available from sources such as the American Type Culture Collection, Rockville, Maryland.

Similarly, a number of strains of isolated human CMV may be employed from which a desired gB subunit is derived. For example, the Towne strain of CMV, a  
35 preferred strain for use in preparation of a vaccine of

this invention because of its broad antigenic spectrum and its attenuation, was isolated from the urine of a two month old male infant with cytomegalic inclusion disease (symptoms - central nervous system damage and  
5 hepatosplenomegaly). This strain of CMV was isolated by Stanley A. Plotkin, M.D. and is described in J. Virol., 11 (6): 991 (1973). This strain is freely available from The Wistar Institute or from the ATCC under accession number VR-977. However, other strains of CMV useful in  
10 the practice of this invention may be obtained from depositories like the ATCC or from other institutes or universities.

In the practice of one embodiment of this invention the HCMV subunit may be produced *in vitro* by  
15 recombinant techniques in large quantities sufficient for use in an immunogenic composition or subunit vaccine. Alternatively, the recombinant adenovirus containing the subunit may itself be employed as an immunogenic or vaccine component, capable of expressing the subunit in  
20 *vivo*.

The presently preferred subunit proteins for use in the present invention are the HCMV gB subunit fragments. One embodiment of the present invention provides a replication competent (non-defective)  
25 adenovirus vector carrying a fragment of the HCMV gB gene which contains a CTL epitope and/or B cell epitope. A preferred gene fragment encodes about amino acid 1 to about amino acid 303 of the gB subunit protein SEQ ID NO:2. Another suitable fragment of gB SEQ ID NO:2 is the  
30 fragment spanning about amino acid 1 to about amino acid 700 of SEQ ID NO:2. Still another suitable gB fragment spans about amino acid 1 to about amino acid 465 of SEQ ID NO:2.

More particularly, it is anticipated that  
35 smaller fragments containing all or a portion of the gB



fragment spanning amino acids about 155 to about 303 will also be desirable for vaccine use. This region is suspected of containing at least a CTL epitope (see Examples 5 and 6 below).

5           It is anticipated that in the construction of the adenovirus vectors of this invention, any of the subunits of the HCMV envelope protein may be employed. In a manner similar to the use of the gB fragment in this vaccine, other subunits of CMV which may be employed in  
10 the production of a vaccine according to the invention may be selected from the gcII, gcIII, or immediate early subunits of the human virus. Alternatively, more than one HCMV subunit may be employed in a vaccine according to the teachings of the present invention.

15           In addition to isolating the desired subunit from an available strain of HCMV for insertion into the selected adenovirus, the sequences of the subunits of two HCMV strains have been published [See, e.g., Mach et al, J. Gen. Virol., 67:1461-1467 (1986); Cranage et al,  
20 (1986) cited above; and Spaete et al, Virol., 167:207-225 (1987). These subunit sequences can therefore be chemically synthesized by conventional methods known to one of skill in the art, or the sequences purchased from commercial sources.

25           The recombinant adenovirus of the present invention may also contain multiple copies of the HCMV subunit. Alternatively, the recombinant virus may contain more than one HCMV subunit type, so that the virus may express two or more HCMV subunits, subunit  
30 fragments, or immediate early antigens and subunits together.

          In the construction of the adenovirus vector of the present invention, the CMV subunit sequence is preferably inserted in an adenovirus strain under the  
35 control of an expression control sequence in the virus

itself. The adenovirus vector of the present invention preferably contains other sequences of interest in addition to the HCMV subunit. Such sequences may include regulatory sequences, enhancers, suitable promoters, secretory signal sequences and the like. The techniques employed to insert the subunit sequence into the adenovirus vector and make other alterations in the viral DNA, e.g., to insert linker sequences and the like, are known to one of skill in the art. See, e.g., T. Maniatis et al, "Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). Thus, given the disclosures contained herein the construction of suitable adenovirus expression vectors for expression of an HCMV subunit protein is within the skill of the art. Example 3 below provides construction details for the non-defective adenovirus containing these gB fragments.

The recombinant adenovirus itself, constructed as described above, may be used directly as an immunogen or a vaccine component. According to this embodiment of the invention, the recombinant adenovirus, containing the HCMV subunit, e.g., the gB subunit fragment, is introduced directly into the patient by vaccination. The recombinant virus, when introduced into a patient directly, infects the patient's cells and produces the CMV subunit in the patient's cells. The inventors have found that this method of presenting these HCMV genes to a vaccinate is particularly capable of eliciting an immune response. Examples 5 and 6 demonstrate the ability of a recombinant adenovirus containing the gB fragment, amino acid 1-303 of SEQ ID NO:2, to induce a gB-specific, protective CTL response in mice.

The use of these adenovirus recombinants as immunogens capable of inducing a CTL response is surprising in view of the results obtained in the same

assays of the examples with other known virus types, which have been used in vaccines previously. According to another embodiment of this invention, once the recombinant viral vector containing the CMV subunit protein, e.g., the gB<sub>1-303</sub> subunit fragment, is constructed, it may be infected into a suitable host cell for *in vitro* expression. The infection of the recombinant viral vector is performed in a conventional manner. [See, Maniatis et al, supra.] Suitable host cells include, without limitation, mammalian cells and cell lines, e.g., A549 (human lung carcinoma) or 293 (transformed human embryonic kidney) cells.

The host cell, once infected with the recombinant virus of the present invention, is then cultured in a suitable medium, such as Minimal Essential Medium (MEM) for mammalian cells. The culture conditions are conventional for the host cell and allow the subunit, e.g., gB<sub>1-303</sub> subunit fragment, to be produced either intracellularly, or secreted extracellularly into the medium. Conventional protein isolation techniques are employed to isolate the expressed subunit from the selected host cell or medium.

When expressed *in vitro* and isolated from culture, the subunit, e.g., gB<sub>1-303</sub>, may then be formulated into an appropriate vaccine composition. Such compositions may generally contain one or more of the recombinant CMV subunits.

The preparation of a pharmaceutically acceptable vaccine composition, having appropriate pH, isotonicity, stability and other conventional characteristics is within the skill of the art. Thus such vaccines may optionally contain other components, such as adjuvants and/or carriers, e.g., aqueous suspensions of aluminum and magnesium hydroxides.

Thus, the present invention also includes a method of vaccinating humans against human CMV infection with the recombinant adenovirus vaccine composition. This vaccine composition is preferably orally  
5 administered, because adenoviruses are known to replicate in cells of the stomach. Previous studies with adenoviruses have shown them to be safe when administered orally [see, e.g., Collis et al, cited above]. However, the present invention is not limited by the route of  
10 administration selected for the vaccine.

When the recombinant adenovirus is administered as the vaccine, a dosage of between  $10^5$  and  $10^8$  plaque forming units may be used. Additional doses of the vaccines of this invention may also be administered where  
15 considered desirable by the physician. The dosage regimen involved in the method for vaccination against CMV infection with the recombinant virus of the present invention can be determined considering various clinical and environmental factors known to affect vaccine  
20 administration.

Alternatively, the vaccine composition may comprise one or more recombinantly-produced human CMV subunit proteins, preferably a fragment of a gB subunit. The *in vitro* produced subunit proteins may be introduced  
25 into the patient in a vaccine composition as described above, preferably employing the oral, nasal or subcutaneous routes of administration. The presence of the subunit produced either *in vivo* or as part of an *in vitro* expressed subunit administered with a carrier,  
30 stimulates an immune response in the patient. Such an immune response is capable of providing protection against exposure to the whole human CMV microorganism. The dosage for all routes of administration of the *in vitro* vaccine containing one or more of the CMV subunit  
35 proteins is generally greater than 20 micrograms of

protein per kg of patient body weight, and preferably between 40 and 80 micrograms of protein per kilogram.

The utility of the recombinant adenoviruses of the present invention is demonstrated through the use of a novel mouse experimental model which characterizes cytotoxic T lymphocyte (CTL) responses to individual proteins of strictly human-restricted viruses. For example, the model as used herein is based on the use of two types of recombinant viruses, an adenovirus and a canarypox virus, both expressing a gene of the same HCMV protein. This model is useful in identifying immunodominant HCMV proteins and immunodominant epitopes of individual proteins to incorporate into an appropriate immunizing vector, analysis of proteins of various HCMV strains, immunization protocols and the longevity of cell-mediated immunity to individual proteins or epitopes; and investigation of the optimal vector for effective introduction of a certain antigen or epitope to the host immune system.

According to this model, mice are immunized with one recombinant of the invention, and CTL activity is tested in target cells infected with the other recombinant. Specifically, Examples 4-6 below provide a murine model of the cytotoxic T lymphocyte (CTL) response to the amino acid 1-303 fragment of the glycoprotein B (gB) gene [SEQ ID NO:2] of human cytomegalovirus (HCMV) based on the use of gB-expressing adenovirus (Ad-gB) and several poxvirus recombinants. Using this model, it has been demonstrated that the human CMV subunit gB (HCMV-gB) amino acid 1-303 fragment can elicit a major histocompatibility complex (MHC) class I-restricted HCMV-gB-specific CTL response in mice.

The following examples illustrate the construction of a non-defective adenovirus strain capable of expressing the HCMV major envelope glycoprotein gB<sub>1-303</sub>

fragment and the efficacy of these compositions as an HCMV vaccine. These examples are illustrative only and do not limit the scope of the present invention.

5    Example 1 - Construction of a Non-defective Adenovirus - gB (Ad-gB) Recombinant

          The gB gene was cloned from the Towne strain of HCMV [Wistar Institute] as follows. The gB gene was first mapped to the 20.5 kb Hind III D fragment of HCMV  
10    using oligonucleotides that corresponded to the 5' and 3' termini of the published AD-169 gB sequence [See, Cranage et al (1986), cited above]. The Hind III fragment was cut with XbaI to generate a 9.8 kb fragment. This fragment was then cut with XmaIII to generate a 3.1 kb  
15    fragment. The 3.1 kb XmaIII fragment which contained the gB gene, had XbaI linkers attached to its 5' and 3' termini.

          An adenovirus type 5 plasmid, pAd5 Bam-B, which contains the 59.5 - 100 mu region of the Ad5 adenovirus  
20    genome cloned into the BamHI site of pBR322 [See, R. L. Berkner et al, Nucl. Acids Res., 11:6003-6020 (1983) and M. E. Morin et al, cited above] was digested with XbaI to remove the 78.5 mu - 84.7 mu sequences of the Ad5 genome. The 78.5 to 84.7 mu deletion removes most of the coding  
25    region of the E3 transcription unit of Ad5 but leaves the E3 promoter intact. The XbaI-linked 3.1 kb fragment of CMV containing the gB gene was inserted into this XbaI site of pAd5 Bam-B. Fig. 1A provides a diagrammatic illustration of the above description.

30           To generate recombinant virus, the 0-76 mu fragment of wild type Ad5 virus was isolated by digesting the viral DNA with EcoRI [See, U. Petterson et al, J. Mol. Biol., 73:125-130 (1973)]. This fragment was co-transfected with the 59.5 to 100 mu BamHI fragment of  
35    pAd5 Bam-B containing the gB gene as described above into

human embryonic kidney 293 cells, available from the American Type Culture Collection. The Ad-gB recombinant was generated by overlap recombination between the viral sequences as illustrated in Fig. 1B.

5           The gB recombinant virus was plaque purified on human lung carcinoma A549 cells [ATCC CCL185] using standard procedures. Viruses containing both orientations of the gB gene, as determined by Southern blotting, were isolated.

10           The recombinant containing the gB gene in the same 5' to 3' direction as the adenovirus E3 promoter of the adenovirus type 5 strain is under the transcriptional control of the E3 promoter. The plaque purified recombinant virus retains the cloning XbaI sites. The  
15   above-described cloned gB gene is devoid of its natural promoter according to the DNA sequence of gB identified in Spaete et al, (1987), cited above.

#### Example 2 - Production of the Full-Length gB Subunit

20           The adenovirus gB plasmid construct and the Ad5 mu 0-76 DNA of Example 1 were cotransfected into 293 cells, human cells transformed by adenovirus 5 early genes [See, Graham et al, J. Gen. Virol., 36:59-72 (1977); and ATCC CRL1573] employing conventional procedures.  
25   This transfection generated a functional recombinant virus by homologous overlap recombination as shown in Fig. 1B.

          Southern blot analysis confirmed the presence of an adenovirus, type 5, containing the HCMV gB subunit  
30   (referred to as either Ad-5/gB or Ad-gB) recombinant virus which was subsequently purified by plaque purification using standard procedures.

          The recombinant virus AD-5/gB, expresses gB subunit protein as determined by conventional assays,  
35   i.e., immunofluorescence on fixed cells and by Western

blot using monospecific guinea pig antiserum and monoclonal antibodies to gB protein [See, e.g., T. Maniatis et al, cited above]. The Ad-5/gB recombinant, also referred to as Ad-gB, is also described in applicant's publication [Marshall et al., J. Infect. Dis., 162:1177-1181 (1990)] published after the filing date of the original parent application from which this application claims priority.

10 Example 3 - Construction of the gB gene fragments

Ad-gB<sub>1-303</sub> and Ad-gB<sub>1-155</sub> recombinant viruses were constructed by overlap recombination as described for Ad-gB in Example 2 above. Briefly, in order to clone the subfragments of the gB gene, five oligonucleotide primers for polymerase chain reactions (PCR) were synthesized. The primers were designed to anneal with various portions of the gB DNA sequence and promote amplification of the gene. In addition, all of the oligonucleotide primers were engineered to contain an Xba I site so that the PCR product could be digested with this enzyme in order to facilitate cloning into the pAd-5 vector.

5' gB primer : SEQ ID NO:3:

4889: 5'-ACACGCAAGAGA TCTAGA CGCGCCTCAT

3' primer at amino acid 700 of gB protein: SEQ ID NO:4:

25 5'-TCGTCCAGAC TCTAGA GGTAGGGC

3' primer at aa 465: SEQ ID NO:5:

5'-CGACTCCAT TCTAGA TTAATGAGTTGCATT

3' primer at aa 303: SEQ ID NO:6:

5'-CAAAGTCGGAG TCTAGAG TCTAGTTCGGAAA

30 3' primer at aa 155: SEQ ID NO:7:

5'-CAGATAAGTGG TCTAGA TCTAAGCGTAGCTACG

The above oligonucleotides correspond to the following nucleotide positions of the HCMV gB gene (Towne strain) as reported by Spaete et al, Virology, 167:207-225

35 (1988). SEQ ID NO:3 corresponds to nucleotide positions



895 to 922 in the sense orientation; SEQ ID NO:4 to nucleotide positions 3090 to 3067 anti-sense; SEQ ID NO:5 to nucleotide positions 2375 to 2350 anti-sense; SEQ ID NO:6 to nucleotide positions 1877 to 1847 anti-sense; and  
5 SEQ ID NO:7 to nucleotide positions 1432 to 1400 anti-sense. These immediately preceding nucleotide numbers are not identical to those of SEQ ID NO: 1 because the Spaete et al sequence, to which these numbers correspond, contains additional 5' non-coding sequence while SEQ ID  
10 NO: 1 reports only the DNA sequence corresponding to the coding region of the gB protein [SEQ ID NO: 2].

The specific segments or fragments of the gB gene were amplified using the Perkin-Elmer Amplitaq™ kit by mixing 400 ng of the 5' gB primer with each of the 3'  
15 primers separately (400 ng of each) and 0.1 µg of purified HCMV genomic DNA or 0.1 µg of previously cloned intact gB gene (see Example 2). The final reaction mixture was 100 µL and the thermocycling conditions were 94°C, 1 minute; 52°C, 1 minute; 72°C, 1 minute, repeated  
20 for a total of 35 cycles. Amplified DNA was purified by cutting the proper DNA fragment out of a 1.2% agarose gel, digested with *Xba*I, repurified by cutting the digested fragments out of a 1.2% agarose gel and then ligated into the *Xba*I site of the cloning vector pAd-5.  
25 Positive recombinants were verified by DNA sequence analysis and sequence analysis confirmed the orientation of the clones.

#### Example 4 - CTL Assays

##### 30 A. Recombinant Viruses Used

The following recombinant viruses were used in the CTL assays of Examples 5-6 below to demonstrate the immunogenicity and vaccine utility of the recombinant adenoviruses of the present invention.

Wild-type human adenovirus type 5 (WT-Ad) and the Ad-gB recombinant were propagated in human lung carcinoma A549 cells [ATCC CCL185], as described in Example 1.

5           An E3-deleted adenovirus type 5 mutant lacking the XbaI D fragment of adenovirus DNA (Ad5ΔE3) was constructed by overlap recombination, using plasmid pAd-5 mu 59.5-100, which was deleted in E3 sequences (mu 78.5-84) using the techniques described in Example 1, and  
10       pAd-5 mu 0-75.9 [G. S. Marshall et al, J. Infect. Dis., 162:1177-1181 (1990), hereby incorporated by reference].

          A vaccinia virus recombinant containing the gB subunits (VacC-gB) described previously in Gonczol et al, Vaccine, 9:631-637 (1991) and the parental Copenhagen  
15       strain of vaccinia, VC-2 (also known as wild-type vaccinia (WT-Vac)) were grown in Vero cells [E. Gonczol et al, Vaccine, 8:130-136 (1990); J. Tartaglia et al, Crit. Rev. Immunol., 10:13-30 (1990)].

          The vaccinia WR strain [obtained from Dr. Enzo Paoletti, Virogenetics Corp, Troy, NY] was used to  
20       develop a recombinant expressing HCMV-gB ((VacW)-gB). This recombinant was derived using a strategy similar to that described for the VacC-gB recombinant (Gonczol et al., cited above).

25           A canarypox recombinant [ALVAC-CMV (vCP139) which is subsequently referred to as Cp-gB] expressing the HCMV-gB gene was constructed using a strategy similar to that described for a canarypox-rabies recombinant in Taylor et al., Vaccine, 9:190-193 (1991) [also obtained  
30       from Dr. Enzo Paoletti]. Briefly, the gene encoding the HCMV (Towne strain) glycoprotein B was inserted into a canarypox donor plasmid consisting of a polylinker flanked by genomic sequence from which a nonessential gene was specifically deleted (at a unique EcoRI site  
35       within a 3.3 kbp PvuII subgenomic fragment of canarypox

DNA). Expression of the gB protein gene was placed under the transcriptional control of an early/late vaccinia virus promoter (H6) previously described [Percus et al., J. Virol., 63:3829-3835 (1989)]. Cp-gB was derived and plaque-purified by standard methods [Panicali and Paoletti, Proc. Natl. Acad. Sci. USA, 79:4927-4931 (1982)]. The Cp-gB recombinant and parental canarypox virus (WT-Cp) were propagated on primary chick embryo fibroblasts.

10           B.   Expression of the gB protein in Cp-gB recombinant virus

Chicken embryo fibroblast (CEF) cells [ATCC CRL 1590] infected with either Cp-gB or with the parental wild-type canarypox (WT-Cp) virus preparations were analyzed by Western blot assay using the 4A guinea-pig serum directed against the gB protein. Western blot assays and the 4A guinea-pig serum, used as gB-specific antibody, were described previously in Gonczol et al., J. Virol., 58:661-664 (1986). Uninfected and HCMV-infected MRC-5 cell [ATCC CCL 171] lysates were included as controls.

A diffuse band at the 140 kDa position and a double band of 55 and 58 kDa were detected in both Cp-gB-infected CEF cells and in HCMV-infected MRC-5 cells. The presence of these gB-specific proteins presumably representing the glycosylated 140 kDa precursor and the differentially glycosylated cleavage products (55 and 58 kDa) indicates that the Cp-gB recombinant expresses the inserted gB gene. The slight difference between the mobility of 55 and 58 kDa cleavage products of control and recombinant gB may reflect different glycosylation patterns.

C.   Murine Model and CTL Assay

For immunization of mice, Ad-gB and WT-Ad were purified by CsCl gradient centrifugation. VacC-gB,

VacW-gB and WT-Vac were purified by sucrose gradient centrifugation, and Cp-gB and WT-Cp were concentrated on sucrose cushion.

Six- to 8-week-old female BALB/c and CBA mice (from Harlan Sprague-Dawley and Jackson) and 12-week-old male BALB/k mice (from The Wistar Institute Animal Facility) were immunized intraperitoneally (i.p.) with the recombinant viruses described above at  $1-5 \times 10^8$  pfu unless otherwise stated.

One to 12 weeks later, spleens were aseptically removed and cell suspensions were prepared by gently pressing the spleens through a stainless steel mesh. Cells were suspended at  $2.5 \times 10^6$  viable cells/ml in RPMI 1640 medium containing 5% FBS (Gibco),  $2 \times 10^{-5}$  M 2-mercaptoethanol, 14 mM HEPES buffer, glutamine and 50  $\mu$ g/ml gentamicin. Spleen cell cultures were restimulated *in vitro* with Ad-gB (multiplicity of infection (m.o.i.) = 10) or VacC-gB (m.o.i. = 0.5) infected autologous spleen cells for 5 days in 24-well plates. Cytolytic activity of nonadherent spleen cells was tested in a chromium release assay which was performed as follows.

1. T-cell subset depletion

For *in vitro* depletion of CD4 or CD8 cells,  $3 \times 10^6$  spleen cells were incubated with anti-mouse CD4 monoclonal antibody (MAb) [Pharmingen; Cat.3:01061 D; 20  $\mu$ g/ $3 \times 10^6$  cells] or CD8 MAb [Accurate; Cat.#:CL-8921; diluted 1:4] for 60 minutes at 4°C, and further incubated in the presence of rabbit complement [Accurate; Low-tox M; diluted 1:10] for 30 minutes at 37°C. The cells were washed twice and used as effector cells in a  $^{51}\text{Cr}$ -release test.

2. Chromium release assay

P815 (H-2<sup>d</sup>) [ATCC TIB 64], mouse MC57 (H-2<sup>b</sup>) cells [also termed MC-57G, D.P. Aden et al, Immunogenetics, 3:209-221 (1976)] and mouse NCTC clone

929 (H-2<sup>k</sup>) cells [ATCC CCL 1] were used as target cells. The HCMV neutralization titer of mouse sera was determined on MRC-5 cells [ATCC CCL 171] by the microneutralization method as described in Gonczol et al., J. Virol. Methods, 14:37-41 (1986).

The target cells were infected with Ad-gB or Ad-5ΔE3 (multiplicity of infection (m.o.i.) = 40-80, 40 hours) or with Vac-gB or WT-Vac (m.o.i. = 5-10, 4 hours). Target cells were washed in the modified RPMI 1640 medium described above and 2 x 10<sup>6</sup> cells were labeled with 100 μCi of [<sup>51</sup>Cr]NaCrO<sub>4</sub> [Amersham, specific activity 250-500 mCi/mg] for 1 hour. The labeled target cells were washed 3 times in phosphate-buffered saline (PBS) and then mixed with the effector cells at various effector:target ratios in triplicate using 96-well U-bottomed microtiter plates and incubated for 4 hours.

Percentage specific <sup>51</sup>Cr release was calculated as: [(cpm experimental release - cpm spontaneous release) / (cpm maximal release - cpm spontaneous release)] x 100. Standard deviation of the mean of triplicate cultures was less than 10%, and spontaneous release was always less than 25%.

This CTL assay is a system in which two types of viral expression vectors, poxvirus and adenovirus, carrying the same fragment of the HCMV-gB gene, are alternately used for immunization of animal or for infection of target cells to show that HCMV-gB fragment is an inducer of CTL in mice. Using this model system, the relative immunogenicity of the gB fragment expressed by different recombinant viruses has been evaluated.

#### Example 5 - CTL Responses to Adenovirus Containing gB Fragments

Ad-gB<sub>1-303</sub> and Ad-gB<sub>1-155</sub> recombinant viruses were constructed as described in Example 3 above.

In CTL experiments performed as described in Example 4, CBA mice were immunized i.p. with  $10^8$  pfu of the Ad-gB, Ad-gB<sub>1-303</sub> or Ad-gB<sub>1-155</sub>. Two weeks later spleen cells were restimulated in vitro with Ad-gB infected autologous spleen cells and tested for ability to lyse Wt-Ad, Vac-gB or Wt-Vac infected L929 (MHC-class I matched) cells.

All recombinants showed an Ad virus-specific CTL response, but only Ad-gB (containing the complete gB coding sequence) and Ad-gB<sub>1-303</sub> exerted gB-specific CTL, indicating the presence of a CTL-epitope on the N-terminal part of the gB protein between amino acid 155 and 303.

#### Example 6 - Protection Studies with Adenovirus Containing gB Fragments

Using the murine model described in Example 4, CBA mice were immunized with  $1 \times 10^8$  pfu of Wt-Ad, Ad5Δ3 (an E3 deleted mutant virus, the parental strain of the recombinant viruses), Ad-gB, Ad-gB<sub>1-303</sub> or Ad-gB<sub>1-155</sub>. Five to ten days later the immunized mice were challenged i.c. with VacWR-gB (a neurovirulent vaccinia strain expressing the HCMV-gB protein). Control mice, immunized with the Wt-Ad or Ad5Δ3 virus died within 4-7 days after the challenge.

Ad-gB and Ad-gB<sub>1-303</sub>-immunized mice survived (92% and 95% survival, respectively), while all of the Ad-gB<sub>1-155</sub>-immunized mice died, indicating a protection epitope on the N-terminal part of the gB protein between amino acid 155 and 303.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. For example, use of other appropriate non-defective adenovirus strains for construction of

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analogous expression systems to express the HCMV gB fragment may be constructed according to the disclosure of the present invention.

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Additionally, the other subunits of HCMV major glycoprotein complexes, e.g., gCII or gCIII, or immediate-early antigens, may be expressed in a non-defective adenovirus recombinant in the same manner as described above for subunit gB fragment. Such  
10 modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Wistar Institute of Anatomy, Biology  
Government of USA Dept.  
Health and Human Services
- (ii) TITLE OF INVENTION: Recombinant Cytomegalovirus  
Vaccine
- (iii) NUMBER OF SEQUENCES: 7
- (iv) CORRESPONDENCE ADDRESS:
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  - (E) COUNTRY: USA
  - (F) ZIP: 19477
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
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  - (C) CLASSIFICATION:
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  - (A) APPLICATION NUMBER: US 08/048,978
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## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2724 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..2721

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG	GAA	TCC	AGG	ATC	TGG	TGC	CTG	GTA	GTC	TGC	GTT	AAC	TTG	42
Met	Glu	Ser	Arg	Ile	Trp	Cys	Leu	Val	Val	Cys	Val	Asn	Leu	
1				5					10					
TGT	ATC	GTC	TGT	CTG	GGT	GCT	GCG	GTT	TCC	TCA	TCT	TCT	ACT	84
Cys	Ile	Val	Cys	Leu	Gly	Ala	Ala	Val	Ser	Ser	Ser	Ser	Thr	
15				20					25					
CGT	GGA	ACT	TCT	GCT	ACT	CAC	AGT	CAC	CAT	TCC	TCT	CAT	ACG	126
Arg	Gly	Thr	Ser	Ala	Thr	His	Ser	His	His	Ser	Ser	His	Thr	
	30					35					40			
ACG	TCT	GCT	GCT	CAT	TCT	CGA	TCC	GGT	TCA	GTC	TCT	CAA	CGC	168
Thr	Ser	Ala	Ala	His	Ser	Arg	Ser	Gly	Ser	Val	Ser	Gln	Arg	
	45					50						55		
GTA	ACT	TCT	TCC	CAA	ACG	GTC	AGC	CAT	GGT	GTT	AAC	GAG	ACC	210
Val	Thr	Ser	Ser	Gln	Thr	Val	Ser	His	Gly	Val	Asn	Glu	Thr	
			60				65					70		
ATC	TAC	AAC	ACT	ACC	CTC	AAG	TAC	GGA	GAT	GTG	GTG	GGG	GTC	252
Ile	Tyr	Asn	Thr	Thr	Leu	Lys	Tyr	Gly	Asp	Val	Val	Gly	Val	
			75				80							
AAC	ACC	ACC	AAG	TAC	CCC	TAT	CGC	GTG	TGT	TCT	ATG	GCA	CAG	294
Asn	Thr	Thr	Lys	Tyr	Pro	Tyr	Arg	Val	Cys	Ser	Met	Ala	Gln	
85					90					95				
GGT	ACG	GAT	CTT	ATT	CGC	TTT	GAA	CGT	AAT	ATC	GTC	TGC	ACC	336
Gly	Thr	Asp	Leu	Ile	Arg	Phe	Glu	Arg	Asn	Ile	Val	Cys	Thr	
100					105						110			
TCG	ATG	AAG	CCC	ATC	AAT	GAA	GAC	CTG	GAC	GAG	GGC	ATC	ATG	378
Ser	Met	Lys	Pro	Ile	Asn	Glu	Asp	Leu	Asp	Glu	Gly	Ile	Met	
	115					120						125		

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GTG	GTC	TAC	AAA	CGC	AAC	ATC	GTC	GCG	CAC	ACC	TTT	AAG	GTA	420
Val	Val	Tyr	Lys	Arg	Asn	Ile	Val	Ala	His	Thr	Phe	Lys	Val	
			130					135					140	
CGA	GTC	TAC	CAG	AAG	GTT	TTG	ACG	TTT	CGT	CGT	AGC	TAC	GCT	462
Arg	Val	Tyr	Gln	Lys	Val	Leu	Thr	Phe	Arg	Arg	Ser	Tyr	Ala	
			145					150						
TAC	ATC	CAC	ACC	ACT	TAT	CTG	CTG	GCG	AGC	AAC	ACG	GAA	TAC	504
Tyr	Ile	His	Thr	Thr	Tyr	Leu	Leu	Gly	Ser	Asn	Thr	Glu	Tyr	
155					160					165				
GTG	GCG	CCT	CCT	ATG	TGG	GAG	ATT	CAT	CAT	ATC	AAC	AGT	CAC	546
Val	Ala	Pro	Pro	Met	Trp	Glu	Ile	His	His	Ile	Asn	Ser	His	
	170					175					180			
AGT	CAG	TGC	TAC	AGT	TCC	TAC	AGC	CGC	GTT	ATA	GCA	GGC	ACG	588
Ser	Gln	Cys	Tyr	Ser	Ser	Tyr	Ser	Arg	Val	Ile	Ala	Gly	Thr	
		185					190					195		
GTT	TTC	GTG	GCT	TAT	CAT	AGG	GAC	AGC	TAT	GAA	AAC	AAA	ACC	630
Val	Phe	Val	Ala	Tyr	His	Arg	Asp	Ser	Tyr	Glu	Asn	Lys	Thr	
			200					205					210	
ATG	CAA	TTA	ATG	CCC	GAC	GAT	TAT	TCC	AAC	ACC	CAC	AGT	ACC	672
Met	Gln	Leu	Met	Pro	Asp	Asp	Tyr	Ser	Asn	Thr	His	Ser	Thr	
				215					220					
CGT	TAC	GTG	ACG	GTC	AAG	GAT	CAA	TGG	CAC	AGC	CGC	GGC	AGC	714
Arg	Tyr	Val	Thr	Val	Lys	Asp	Gln	Trp	His	Ser	Arg	Gly	Ser	
225					230					235				
ACC	TGG	CTC	TAT	CGT	GAG	ACC	TGT	AAT	CTG	AAT	TGT	ATG	GTG	756
Thr	Trp	Leu	Tyr	Arg	Glu	Thr	Cys	Asn	Leu	Asn	Cys	Met	Val	
	240					245					250			
ACC	ATC	ACT	ACT	GCG	CGC	TCC	AAG	TAT	CCC	TAT	CAT	TTT	TTC	798
Thr	Ile	Thr	Thr	Ala	Arg	Ser	Lys	Tyr	Pro	Tyr	His	Phe	Phe	
		255					260					265		
GCA	ACT	TCC	ACG	GGT	GAT	GTG	GTT	GAC	ATT	TCT	CCT	TTC	TAC	840
Ala	Thr	Ser	Thr	Gly	Asp	Val	Val	Asp	Ile	Ser	Pro	Phe	Tyr	
			270					275					280	
AAC	GGA	ACT	AAT	CGC	AAT	GCC	AGC	TAT	TTT	GGA	GAA	AAC	GCC	882
Asn	Gly	Thr	Asn	Arg	Asn	Ala	Ser	Tyr	Phe	Gly	Glu	Asn	Ala	
				285					290					
GAC	AAG	TTT	TTC	ATT	TTT	CCG	AAC	TAC	ACT	ATC	GTC	TCC	GAC	924
Asp	Lys	Phe	Phe	Ile	Phe	Pro	Asn	Tyr	Thr	Ile	Val	Ser	Asp	
295					300					305				

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TTT	GGA	AGA	CCG	AAT	TCT	GCG	TTA	GAG	ACC	CAC	AGG	TTG	GTG	966
Phe	Gly	Arg	Pro	Asn	Ser	Ala	Leu	Glu	Thr	His	Arg	Leu	Val	
	310					315					320			
GCT	TTT	CTT	GAA	CGT	GCG	GAC	TCA	GTG	ATC	TCC	TGG	GAT	ATA	1008
Ala	Phe	Leu	Glu	Arg	Ala	Asp	Ser	Val	Ile	Ser	Trp	Asp	Ile	
		325					330					335		
CAG	GAC	GAG	AAG	AAT	GTT	ACT	TGT	CAA	CTC	ACT	TTC	TGG	GAA	1050
Gln	Asp	Glu	Lys	Asn	Val	Thr	Cys	Gln	Leu	Thr	Phe	Trp	Glu	
			340					345					350	
GCC	TCG	GAA	CGC	ACC	ATT	CGT	TCC	GAA	GCC	GAG	GAC	TCG	TAT	1092
Ala	Ser	Glu	Arg	Thr	Ile	Arg	Ser	Glu	Ala	Glu	Asp	Ser	Tyr	
				355					360					
CAC	TTT	TCT	TCT	GCC	AAA	ATG	ACC	GCC	ACT	TTC	TTA	TCT	AAG	1134
His	Phe	Ser	Ser	Ala	Lys	Met	Thr	Ala	Thr	Phe	Leu	Ser	Lys	
	365				370					375				
AAG	CAA	GAG	GTG	AAC	ATG	TCC	GAC	TCT	GCG	CTG	GAC	TGT	GTA	1176
Lys	Gln	Glu	Val	Asn	Met	Ser	Asp	Ser	Ala	Leu	Asp	Cys	Val	
	380					385					390			
CGT	GAT	GAG	GCC	ATA	AAT	AAG	TTA	CAG	CAG	ATT	TTC	AAT	ACT	1218
Arg	Asp	Glu	Ala	Ile	Asn	Lys	Leu	Gln	Gln	Ile	Phe	Asn	Thr	
		395					400					405		
TCA	TAC	AAT	CAA	ACA	TAT	GAA	AAA	TAT	GGA	AAC	GTG	TCC	GTC	1260
Ser	Tyr	Asn	Gln	Thr	Tyr	Glu	Lys	Tyr	Gly	Asn	Val	Ser	Val	
			410					415					420	
TTT	GAA	ACC	ACT	GGT	GGT	TTG	GTG	GTG	TTC	TGG	CAA	GGT	ATC	1302
Phe	Glu	Thr	Thr	Gly	Gly	Leu	Val	Val	Phe	Trp	Gln	Gly	Ile	
				425					430					
AAG	CAA	AAA	TCT	CTG	GTG	GAA	CTC	GAA	CGT	TTG	GCC	AAC	CGC	1344
Lys	Gln	Lys	Ser	Leu	Val	Glu	Leu	Glu	Arg	Leu	Ala	Asn	Arg	
	435				440					445				
TCC	AGT	CTG	AAT	CTT	ACT	CAT	AAT	AGA	ACC	AAA	AGA	AGT	ACA	1386
Ser	Ser	Leu	Asn	Leu	Thr	His	Asn	Arg	Thr	Lys	Arg	Ser	Thr	
	450					455					460			
GAT	GGC	AAC	AAT	GCA	ACT	CAT	TTA	TCC	AAC	ATG	GAG	TCG	GTG	1428
Asp	Gly	Asn	Asn	Ala	Thr	His	Leu	Ser	Asn	Met	Glu	Ser	Val	
		465					470					475		
CAC	AAT	CTG	GTC	TAC	GCC	CAG	CTG	CAG	TTC	ACC	TAT	GAC	ACG	1470
His	Asn	Leu	Val	Tyr	Ala	Gln	Leu	Gln	Phe	Thr	Tyr	Asp	Thr	
			480					485					490	

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TTG	CGC	GGT	TAC	ATC	AAC	CGG	GCG	CTG	GCG	CAA	ATC	GCA	GAA	1512
Leu	Arg	Gly	Tyr	Ile	Asn	Arg	Ala	Leu	Ala	Gln	Ile	Ala	Glu	
				495					500					
GCC	TGG	TGT	GTG	GAT	CAA	CGG	CGC	ACC	CTA	GAG	GTC	TTC	AAG	1554
Ala	Trp	Cys	Val	Asp	Gln	Arg	Arg	Thr	Leu	Glu	Val	Phe	Lys	
505					510					515				
GAA	CTT	AGC	AAG	ATC	AAC	CCG	TCA	GCT	ATT	CTC	TCG	GCC	ATC	1596
Glu	Leu	Ser	Lys	Ile	Asn	Pro	Ser	Ala	Ile	Leu	Ser	Ala	Ile	
	520					525					530			
TAC	AAC	AAA	CCG	ATT	GCC	GCG	CGT	TTC	ATG	GGT	GAT	GTC	CTG	1638
Tyr	Asn	Lys	Pro	Ile	Ala	Ala	Arg	Phe	Met	Gly	Asp	Val	Leu	
		535					540					545		
GGT	CTG	GCC	AGC	TGC	GTG	ACC	ATT	AAC	CAA	ACC	AGC	GTC	AAG	1680
Gly	Leu	Ala	Ser	Cys	Val	Thr	Ile	Asn	Gln	Thr	Ser	Val	Lys	
			550					555					560	
GTG	CTG	CGT	GAT	ATG	AAT	GTG	AAG	GAA	TCG	CCA	GGA	CGC	TGC	1722
Val	Leu	Arg	Asp	Met	Asn	Val	Lys	Glu	Ser	Pro	Gly	Arg	Cys	
				565					570					
TAC	TCA	CGA	CCA	GTG	GTC	ATC	TTT	AAT	TTC	GCC	AAC	AGC	TCG	1764
Tyr	Ser	Arg	Pro	Val	Val	Ile	Phe	Asn	Phe	Ala	Asn	Ser	Ser	
575					580					585				
TAC	GTG	CAG	TAC	GGT	CAA	CTG	GGC	GAG	GAT	AAC	GAA	ATC	CTG	1806
Tyr	Val	Gln	Tyr	Gly	Gln	Leu	Gly	Glu	Asp	Asn	Glu	Ile	Leu	
	590					595					600			
TTG	GGC	AAC	CAC	CGC	ACT	GAG	GAA	TGT	CAG	CTT	CCC	AGC	CTC	1848
Leu	Gly	Asn	His	Arg	Thr	Glu	Glu	Cys	Gln	Leu	Pro	Ser	Leu	
		605					610					615		
AAG	ATC	TTC	ATC	GCC	GGC	AAC	TCG	GCC	TAC	GAG	TAC	GTG	GAC	1890
Lys	Ile	Phe	Ile	Ala	Gly	Asn	Ser	Ala	Tyr	Glu	Tyr	Val	Asp	
			620					625					630	
TAC	CTC	TTC	AAA	CGC	ATG	ATT	GAC	CTC	AGC	AGC	ATC	TCC	ACC	1932
Tyr	Leu	Phe	Lys	Arg	Met	Ile	Asp	Leu	Ser	Ser	Ile	Ser	Thr	
				635					640					
GTC	GAC	AGC	ATG	ATC	GCC	CTA	GAC	ATC	GAC	CCG	CTG	GAA	AAC	1974
Val	Asp	Ser	Met	Ile	Ala	Leu	Asp	Ile	Asp	Pro	Leu	Glu	Asn	
645					650					655				
ACC	GAC	TTC	AGG	GTA	CTG	GAA	CTT	TAC	TCG	CAG	AAA	GAA	TTG	2016
Thr	Asp	Phe	Arg	Val	Leu	Glu	Leu	Tyr	Ser	Gln	Lys	Glu	Leu	
	660					665					670			

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CGT	TCC	AGC	AAC	GTT	TTT	GAT	CTC	GAG	GAG	ATC	ATG	CGC	GAG	2058
Arg	Ser	Ser	Asn	Val	Phe	Asp	Leu	Glu	Glu	Ile	Met	Arg	Glu	
		675					680					685		
TTC	AAT	TCG	TAT	AAG	CAG	CGG	GTA	AAG	TAC	GTG	GAG	GAC	AAG	2100
Phe	Asn	Ser	Tyr	Lys	Gln	Arg	Val	Lys	Tyr	Val	Glu	Asp	Lys	
			690					695					700	
GTA	GTC	GAC	CCG	CTG	CCG	CCC	TAC	CTC	AAG	GGT	CTG	GAC	GAC	2142
Val	Val	Asp	Pro	Leu	Pro	Pro	Tyr	Leu	Lys	Gly	Leu	Asp	Asp	
				705					710					
CTC	ATG	AGC	GGC	CTG	GGC	GCC	GCG	GGA	AAG	GCC	GTT	GGC	GTA	2184
Leu	Met	Ser	Gly	Leu	Gly	Ala	Ala	Gly	Lys	Ala	Val	Gly	Val	
715					720					725				
GCC	ATT	GGG	GCC	GTG	GGT	GGC	GCG	GTG	GCC	TCC	GTG	GTC	GAA	2226
Ala	Ile	Gly	Ala	Val	Gly	Gly	Ala	Val	Ala	Ser	Val	Val	Glu	
	730					735					740			
GGC	GTT	GCC	ACC	TTC	CTC	AAA	AAC	CCC	TTC	GGA	GCC	TTC	ACC	2268
Gly	Val	Ala	Thr	Phe	Leu	Lys	Asn	Pro	Phe	Gly	Ala	Phe	Thr	
		745					750					755		
ATC	ATC	CTC	GTG	GCC	ATA	GCC	GTC	GTC	ATT	ATC	ATT	TAT	TTG	2310
Ile	Ile	Leu	Val	Ala	Ile	Ala	Val	Val	Ile	Ile	Ile	Tyr	Leu	
			760					765					770	
ATC	TAT	ACT	CGA	CAG	CGG	CGT	CTC	TGC	ATG	CAG	CCG	CTG	CAG	2352
Ile	Tyr	Thr	Arg	Gln	Arg	Arg	Leu	Cys	Met	Gln	Pro	Leu	Gln	
				775					780					
AAC	CTC	TTT	CCC	TAT	CTG	GTG	TCC	GCC	GAC	GGG	ACC	ACC	GTG	2394
Asn	Leu	Phe	Pro	Tyr	Leu	Val	Ser	Ala	Asp	Gly	Thr	Thr	Val	
785					790					795				
ACG	TCG	GGC	AAC	ACC	AAA	GAC	ACG	TCG	TTA	CAG	GCT	CCG	CCT	2436
Thr	Ser	Gly	Asn	Thr	Lys	Asp	Thr	Ser	Leu	Gln	Ala	Pro	Pro	
	800					805					810			
TCC	TAC	GAG	GAA	AGT	GTT	TAT	AAT	TCT	GGT	CGC	AAA	GGA	CCG	2478
Ser	Tyr	Glu	Glu	Ser	Val	Tyr	Asn	Ser	Gly	Arg	Lys	Gly	Pro	
		815					820					825		
GGA	CCA	CCG	TCG	TCT	GAT	GCA	TCC	ACG	GCG	GCT	CCG	CCT	TAC	2520
Gly	Pro	Pro	Ser	Ser	Asp	Ala	Ser	Thr	Ala	Ala	Pro	Pro	Tyr	
			830					835					840	
ACC	AAC	GAG	CAG	GCT	TAC	CAG	ATG	CTT	CTG	GCC	CTG	GTC	CGT	2562
Thr	Asn	Glu	Gln	Ala	Tyr	Gln	Met	Leu	Leu	Ala	Leu	Val	Arg	
				845					850					

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CTG	GAC	GCA	GAG	CAG	CGA	GCG	CAG	CAG	AAC	GGT	ACA	GAT	TCT	2604
Leu	Asp	Ala	Glu	Gln	Arg	Ala	Gln	Gln	Asn	Gly	Thr	Asp	Ser	
855					860					865				
TTG	GAC	GGA	CAG	ACT	GGC	ACG	CAG	GAC	AAG	GGA	CAG	AAG	CCC	2646
Leu	Asp	Gly	Gln	Thr	Gly	Thr	Gln	Asp	Lys	Gly	Gln	Lys	Pro	
870					875						880			
AAC	CTG	CTA	GAC	CGA	CTG	CGA	CAC	CGC	AAA	AAC	GGC	TAC	CGA	2688
Asn	Leu	Leu	Asp	Arg	Leu	Arg	His	Arg	Lys	Asn	Gly	Tyr	Arg	
		885					890					895		
CAC	TTG	AAA	GAC	TCC	GAC	GAA	GAA	GAG	AAC	GTC	TGA			2724
His	Leu	Lys	Asp	Ser	Asp	Glu	Glu	Glu	Asn	Val				
			900				905							

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 907 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Glu	Ser	Arg	Ile	Trp	Cys	Leu	Val	Val	Cys	Val	Asn	Leu	Cys	Ile
1				5					10					15	
Val	Cys	Leu	Gly	Ala	Ala	Val	Ser	Ser	Ser	Ser	Thr	Arg	Gly	Thr	Ser
			20					25					30		
Ala	Thr	His	Ser	His	His	Ser	Ser	His	Thr	Thr	Ser	Ala	Ala	His	Ser
		35					40					45			
Arg	Ser	Gly	Ser	Val	Ser	Gln	Arg	Val	Thr	Ser	Ser	Gln	Thr	Val	Ser
		50				55					60				
His	Gly	Val	Asn	Glu	Thr	Ile	Tyr	Asn	Thr	Thr	Leu	Lys	Tyr	Gly	Asp
65					70					75				80	
Val	Val	Gly	Val	Asn	Thr	Thr	Lys	Tyr	Pro	Tyr	Arg	Val	Cys	Ser	Met
				85					90					95	
Ala	Gln	Gly	Thr	Asp	Leu	Ile	Arg	Phe	Glu	Arg	Asn	Ile	Val	Cys	Thr
			100					105					110		
Ser	Met	Lys	Pro	Ile	Asn	Glu	Asp	Leu	Asp	Glu	Gly	Ile	Met	Val	Val
		115					120					125			

Tyr	Lys	Arg	Asn	Ile	Val	Ala	His	Thr	Phe	Lys	Val	Arg	Val	Tyr	Gln
130						135					140				
Lys	Val	Leu	Thr	Phe	Arg	Arg	Ser	Tyr	Ala	Tyr	Ile	His	Thr	Thr	Tyr
145					150					155					160
Leu	Leu	Gly	Ser	Asn	Thr	Glu	Tyr	Val	Ala	Pro	Pro	Met	Trp	Glu	Ile
				165					170					175	
His	His	Ile	Asn	Ser	His	Ser	Gln	Cys	Tyr	Ser	Ser	Tyr	Ser	Arg	Val
			180					185					190		
Ile	Ala	Gly	Thr	Val	Phe	Val	Ala	Tyr	His	Arg	Asp	Ser	Tyr	Glu	Asn
		195					200					205			
Lys	Thr	Met	Gln	Leu	Met	Pro	Asp	Asp	Tyr	Ser	Asn	Thr	His	Ser	Thr
	210					215					220				
Arg	Tyr	Val	Thr	Val	Lys	Asp	Gln	Trp	His	Ser	Arg	Gly	Ser	Thr	Trp
225					230					235					240
Leu	Tyr	Arg	Glu	Thr	Cys	Asn	Leu	Asn	Cys	Met	Val	Thr	Ile	Thr	Thr
				245					250					255	
Ala	Arg	Ser	Lys	Tyr	Pro	Tyr	His	Phe	Phe	Ala	Thr	Ser	Thr	Gly	Asp
			260					265					270		
Val	Val	Asp	Ile	Ser	Pro	Phe	Tyr	Asn	Gly	Thr	Asn	Arg	Asn	Ala	Ser
		275					280					285			
Tyr	Phe	Gly	Glu	Asn	Ala	Asp	Lys	Phe	Phe	Ile	Phe	Pro	Asn	Tyr	Thr
	290					295					300				
Ile	Val	Ser	Asp	Phe	Gly	Arg	Pro	Asn	Ser	Ala	Leu	Glu	Thr	His	Arg
305					310					315					320
Leu	Val	Ala	Phe	Leu	Glu	Arg	Ala	Asp	Ser	Val	Ile	Ser	Trp	Asp	Ile
				325					330					335	
Gln	Asp	Glu	Lys	Asn	Val	Thr	Cys	Gln	Leu	Thr	Phe	Trp	Glu	Ala	Ser
			340					345					350		
Glu	Arg	Thr	Ile	Arg	Ser	Glu	Ala	Glu	Asp	Ser	Tyr	His	Phe	Ser	Ser
		355					360					365			
Ala	Lys	Met	Thr	Ala	Thr	Phe	Leu	Ser	Lys	Lys	Gln	Glu	Val	Asn	Met
	370					375					380				
Ser	Asp	Ser	Ala	Leu	Asp	Cys	Val	Arg	Asp	Glu	Ala	Ile	Asn	Lys	Leu
385					390					395					400

30

Gln	Gln	Ile	Phe	Asn	Thr	Ser	Tyr	Asn	Gln	Thr	Tyr	Glu	Lys	Tyr	Gly			
				405					410					415				
Asn	Val	Ser	Val	Phe	Glu	Thr	Thr	Gly	Gly	Leu	Val	Val	Phe	Trp	Gln			
			420					425					430					
Gly	Ile	Lys	Gln	Lys	Ser	Leu	Val	Glu	Leu	Glu	Arg	Leu	Ala	Asn	Arg			
		435					440					445						
Ser	Ser	Leu	Asn	Leu	Thr	His	Asn	Arg	Thr	Lys	Arg	Ser	Thr	Asp	Gly			
	450					455					460							
Asn	Asn	Ala	Thr	His	Leu	Ser	Asn	Met	Glu	Ser	Val	His	Asn	Leu	Val			
465					470					475					480			
Tyr	Ala	Gln	Leu	Gln	Phe	Thr	Tyr	Asp	Thr	Leu	Arg	Gly	Tyr	Ile	Asn			
				485					490					495				
Arg	Ala	Leu	Ala	Gln	Ile	Ala	Glu	Ala	Trp	Cys	Val	Asp	Gln	Arg	Arg			
			500					505					510					
Thr	Leu	Glu	Val	Phe	Lys	Glu	Leu	Ser	Lys	Ile	Asn	Pro	Ser	Ala	Ile			
		515					520					525						
Leu	Ser	Ala	Ile	Tyr	Asn	Lys	Pro	Ile	Ala	Ala	Arg	Phe	Met	Gly	Asp			
	530					535					540							
Val	Leu	Gly	Leu	Ala	Ser	Cys	Val	Thr	Ile	Asn	Gln	Thr	Ser	Val	Lys			
545					550					555					560			
Val	Leu	Arg	Asp	Met	Asn	Val	Lys	Glu	Ser	Pro	Gly	Arg	Cys	Tyr	Ser			
				565					570					575				
Arg	Pro	Val	Val	Ile	Phe	Asn	Phe	Ala	Asn	Ser	Ser	Tyr	Val	Gln	Tyr			
			580					585					590					
Gly	Gln	Leu	Gly	Glu	Asp	Asn	Glu	Ile	Leu	Leu	Gly	Asn	His	Arg	Thr			
		595					600					605						
Glu	Glu	Cys	Gln	Leu	Pro	Ser	Leu	Lys	Ile	Phe	Ile	Ala	Gly	Asn	Ser			
	610					615					620							
Ala	Tyr	Glu	Tyr	Val	Asp	Tyr	Leu	Phe	Lys	Arg	Met	Ile	Asp	Leu	Ser			
625					630					635				640				
Ser	Ile	Ser	Thr	Val	Asp	Ser	Met	Ile	Ala	Leu	Asp	Ile	Asp	Pro	Leu			
				645					650					655				
Glu	Asn	Thr	Asp	Phe	Arg	Val	Leu	Glu	Leu	Tyr	Ser	Gln	Lys	Glu	Leu			
			660					665					670					



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Arg	Ser	Ser	Asn	Val	Phe	Asp	Leu	Glu	Glu	Ile	Met	Arg	Glu	Phe	Asn
		675					680					685			
Ser	Tyr	Lys	Gln	Arg	Val	Lys	Tyr	Val	Glu	Asp	Lys	Val	Val	Asp	Pro
	690					695					700				
Leu	Pro	Pro	Tyr	Leu	Lys	Gly	Leu	Asp	Asp	Leu	Met	Ser	Gly	Leu	Gly
705					710					715					720
Ala	Ala	Gly	Lys	Ala	Val	Gly	Val	Ala	Ile	Gly	Ala	Val	Gly	Gly	Ala
				725					730					735	
Val	Ala	Ser	Val	Val	Glu	Gly	Val	Ala	Thr	Phe	Leu	Lys	Asn	Pro	Phe
			740					745					750		
Gly	Ala	Phe	Thr	Ile	Ile	Leu	Val	Ala	Ile	Ala	Val	Val	Ile	Ile	Ile
		755				760						765			
Tyr	Leu	Ile	Tyr	Thr	Arg	Gln	Arg	Arg	Leu	Cys	Met	Gln	Pro	Leu	Gln
	770					775					780				
Asn	Leu	Phe	Pro	Tyr	Leu	Val	Ser	Ala	Asp	Gly	Thr	Thr	Val	Thr	Ser
785					790					795					800
Gly	Asn	Thr	Lys	Asp	Thr	Ser	Leu	Gln	Ala	Pro	Pro	Ser	Tyr	Glu	Glu
				805					810					815	
Ser	Val	Tyr	Asn	Ser	Gly	Arg	Lys	Gly	Pro	Gly	Pro	Pro	Ser	Ser	Asp
			820					825					830		
Ala	Ser	Thr	Ala	Ala	Pro	Pro	Tyr	Thr	Asn	Glu	Gln	Ala	Tyr	Gln	Met
		835					840					845			
Leu	Leu	Ala	Leu	Val	Arg	Leu	Asp	Ala	Glu	Gln	Arg	Ala	Gln	Gln	Asn
	850					855					860				
Gly	Thr	Asp	Ser	Leu	Asp	Gly	Gln	Thr	Gly	Thr	Gln	Asp	Lys	Gly	Gln
865					870					875					880
Lys	Pro	Asn	Leu	Leu	Asp	Arg	Leu	Arg	His	Arg	Lys	Asn	Gly	Tyr	Arg
				885					890					895	
His	Leu	Lys	Asp	Ser	Asp	Glu	Glu	Glu	Asn	Val					
			900					905							

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## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACACGCAAGA GATCTAGACG CGCCTCAT

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## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCGTCCAGAC TCTAGAGGTA GGGC

24

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGACTCCATT CTAGATTAAT GAGTTGCATT

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## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAAAGTCGGA GTCTAGAGTC TAGTTCGGAA A

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## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 33 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGATAAGTG GTCTAGATCT AAGCGTAGCT ACG

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## WHAT IS CLAIMED IS:

1. A non-defective recombinant adenovirus containing a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence, said virus capable of expressing said subunit protein.

2. The adenovirus according to claim 1 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

3. An immunogenic composition comprising a non-defective recombinant adenovirus and a suitable pharmaceutical carrier, wherein said recombinant adenovirus comprises a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence and said virus is capable of expressing said subunit protein *in vivo* in an animal.

4. The composition according to claim 3 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

5. The composition according to claim 3 wherein said protein gene encodes an additional cytomegalovirus subunit protein fragment or a selected cytomegalovirus subunit protein.

6. The composition according to claim 3 wherein said gB subunit fragment is about amino acid 1 to about amino acid 303 of SEQ ID NO:2.

7. The composition according to claim 3 wherein said adenovirus is selected from the group consisting of an adenovirus type 5, adenovirus type 4 and adenovirus type 7 strain.

8. The composition according to claim 7 wherein said gB subunit fragment is obtained from the Towne strain cytomegalovirus, and the adenovirus is type 5.

9. The use of a non-defective recombinant adenovirus comprising a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence and said virus being capable of expressing said subunit protein *in vivo* in an animal, in the preparation of a CMV vaccine.

10. The use according to claim 9 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

11. The use according to claim 9 wherein said adenovirus is present in an effective amount of between  $10^5$  to  $10^8$  plaque forming units.

12. An immunogenic composition comprising a gB subunit protein fragment containing at least one CTL epitope expressed in a recombinant adenovirus vector.

13. The composition according to claim 12 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

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FIG. 1A

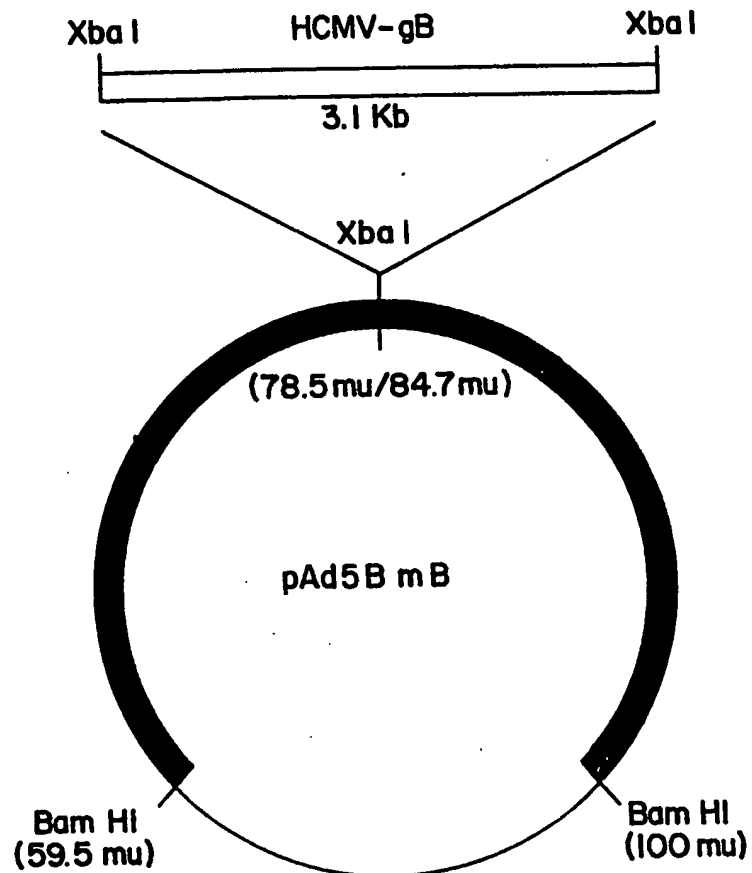
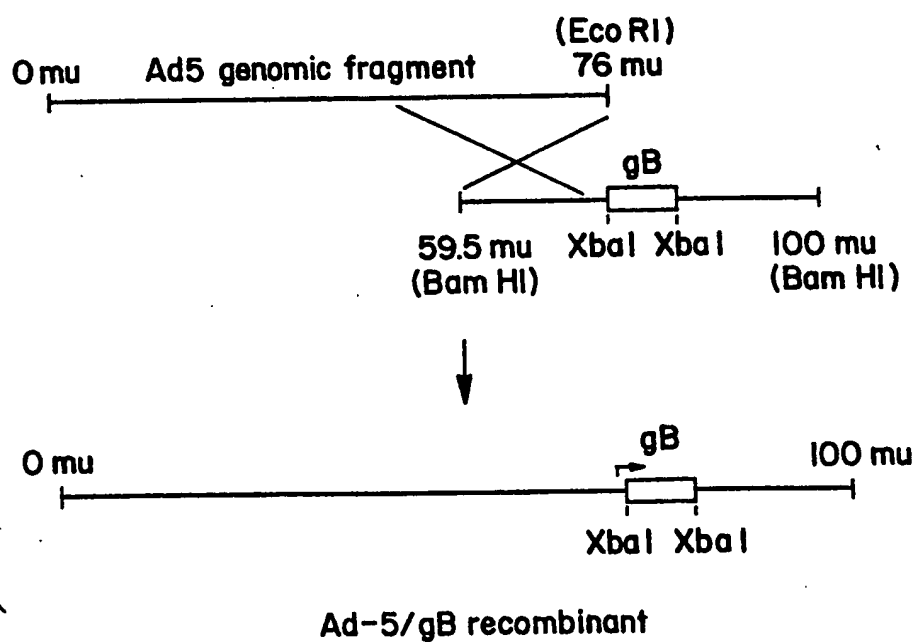


FIG. 1B





## INTERNATIONAL SEARCH REPORT

International application No.

PCT/ISA/NA:88

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : Please See Extra Sheet.

US CL : 424/88, 89; 435/235.1, 69.3, 5, 69.1, 172.3; 436/543; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 89; 435/235.1, 69.3, 5, 69.1, 172.3; 436/543; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

INTELLIGENETICS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	THE EMBO JOURNAL, Volume 5, No. 11, issued November 1986, Cranage et al., "Identification Of The Human Cytomegalovirus Glycoprotein B Gene and Induction of Neutralizing Antibodies via Its Expression in Recombinant Vaccinia Virus", pages 3057-3063, see entire document.	1-13
Y	US, A, 4,920,209 (DAVIS ET AL.) 24 April 1990, see entire document.	1-13



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents:

"A"

document defining the general state of the art which is not considered to be of particular relevance

"E"

earlier document published on or after the international filing date

"L"

document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (to be specified)

"O"

document referring to an oral disclosure, use, exhibition or other means

"P"

document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application text cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"A"

document member of the same patent family

Date of the actual completion of the international search

22 JULY 1994

Date of mailing of the international search report

02 AUG 1994

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

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Authorized officer

LAURIE SCHEINER

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**A. CLASSIFICATION OF SUBJECT MATTER:**

IPC (5):

A61K 39/00, 39/12; C12N 7/00, 15/00; C12P 21/06; C12Q 1/70; G01N 33/531; A01N 43/04

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Tyr	Lys	Arg	Asn	Ile	Val	Ala	His	Thr	Phe	Lys	Val	Arg	Val	Tyr	Gln
130						135					140				
Lys	Val	Leu	Thr	Phe	Arg	Arg	Ser	Tyr	Ala	Tyr	Ile	His	Thr	Thr	Tyr
145					150					155					160
Leu	Leu	Gly	Ser	Asn	Thr	Glu	Tyr	Val	Ala	Pro	Pro	Met	Trp	Glu	Ile
				165					170					175	
His	His	Ile	Asn	Ser	His	Ser	Gln	Cys	Tyr	Ser	Ser	Tyr	Ser	Arg	Val
			180					185						190	
Ile	Ala	Gly	Thr	Val	Phe	Val	Ala	Tyr	His	Arg	Asp	Ser	Tyr	Glu	Asn
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Lys	Thr	Met	Gln	Leu	Met	Pro	Asp	Asp	Tyr	Ser	Asn	Thr	His	Ser	Thr
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Arg	Tyr	Val	Thr	Val	Lys	Asp	Gln	Trp	His	Ser	Arg	Gly	Ser	Thr	Trp
225					230					235					240
Leu	Tyr	Arg	Glu	Thr	Cys	Asn	Leu	Asn	Cys	Met	Val	Thr	Ile	Thr	Thr
				245					250					255	
Ala	Arg	Ser	Lys	Tyr	Pro	Tyr	His	Phe	Phe	Ala	Thr	Ser	Thr	Gly	Asp
			260					265						270	
Val	Val	Asp	Ile	Ser	Pro	Phe	Tyr	Asn	Gly	Thr	Asn	Arg	Asn	Ala	Ser
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Tyr	Phe	Gly	Glu	Asn	Ala	Asp	Lys	Phe	Phe	Ile	Phe	Pro	Asn	Tyr	Thr
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Ile	Val	Ser	Asp	Phe	Gly	Arg	Pro	Asn	Ser	Ala	Leu	Glu	Thr	His	Arg
305					310					315					320
Leu	Val	Ala	Phe	Leu	Glu	Arg	Ala	Asp	Ser	Val	Ile	Ser	Trp	Asp	Ile
				325					330					335	
Gln	Asp	Glu	Lys	Asn	Val	Thr	Cys	Gln	Leu	Thr	Phe	Trp	Glu	Ala	Ser
			340					345					350		
Glu	Arg	Thr	Ile	Arg	Ser	Glu	Ala	Glu	Asp	Ser	Tyr	His	Phe	Ser	Ser
		355					360					365			
Ala	Lys	Met	Thr	Ala	Thr	Phe	Leu	Ser	Lys	Lys	Gln	Glu	Val	Asn	Met
	370					375					380				
Ser	Asp	Ser	Ala	Leu	Asp	Cys	Val	Arg	Asp	Glu	Ala	Ile	Asn	Lys	Leu
385					390					395					400

30

Gln	Gln	Ile	Phe	Asn	Thr	Ser	Tyr	Asn	Gln	Thr	Tyr	Glu	Lys	Tyr	Gly
				405					410					415	
Asn	Val	Ser	Val	Phe	Glu	Thr	Thr	Gly	Gly	Leu	Val	Val	Phe	Trp	Gln
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Gly	Ile	Lys	Gln	Lys	Ser	Leu	Val	Glu	Leu	Glu	Arg	Leu	Ala	Asn	Arg
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Ser	Ser	Leu	Asn	Leu	Thr	His	Asn	Arg	Thr	Lys	Arg	Ser	Thr	Asp	Gly
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Asn	Asn	Ala	Thr	His	Leu	Ser	Asn	Met	Glu	Ser	Val	His	Asn	Leu	Val
465					470					475					480
Tyr	Ala	Gln	Leu	Gln	Phe	Thr	Tyr	Asp	Thr	Leu	Arg	Gly	Tyr	Ile	Asn
				485					490					495	
Arg	Ala	Leu	Ala	Gln	Ile	Ala	Glu	Ala	Trp	Cys	Val	Asp	Gln	Arg	Arg
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Thr	Leu	Glu	Val	Phe	Lys	Glu	Leu	Ser	Lys	Ile	Asn	Pro	Ser	Ala	Ile
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Val	Leu	Gly	Leu	Ala	Ser	Cys	Val	Thr	Ile	Asn	Gln	Thr	Ser	Val	Lys
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Arg	Pro	Val	Val	Ile	Phe	Asn	Phe	Ala	Asn	Ser	Ser	Tyr	Val	Gln	Tyr
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Gly	Gln	Leu	Gly	Glu	Asp	Asn	Glu	Ile	Leu	Leu	Gly	Asn	His	Arg	Thr
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Glu	Glu	Cys	Gln	Leu	Pro	Ser	Leu	Lys	Ile	Phe	Ile	Ala	Gly	Asn	Ser
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Ala	Tyr	Glu	Tyr	Val	Asp	Tyr	Leu	Phe	Lys	Arg	Met	Ile	Asp	Leu	Ser
625					630					635					640
Ser	Ile	Ser	Thr	Val	Asp	Ser	Met	Ile	Ala	Leu	Asp	Ile	Asp	Pro	Leu
				645					650					655	
Glu	Asn	Thr	Asp	Phe	Arg	Val	Leu	Glu	Leu	Tyr	Ser	Gln	Lys	Glu	Leu
			660					665					670		

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Arg	Ser	Ser	Asn	Val	Phe	Asp	Leu	Glu	Glu	Ile	Met	Arg	Glu	Phe	Asn
		675					680					685			
Ser	Tyr	Lys	Gln	Arg	Val	Lys	Tyr	Val	Glu	Asp	Lys	Val	Val	Asp	Pro
	690					695					700				
Leu	Pro	Pro	Tyr	Leu	Lys	Gly	Leu	Asp	Asp	Leu	Met	Ser	Gly	Leu	Gly
705					710					715					720
Ala	Ala	Gly	Lys	Ala	Val	Gly	Val	Ala	Ile	Gly	Ala	Val	Gly	Gly	Ala
				725					730					735	
Val	Ala	Ser	Val	Val	Glu	Gly	Val	Ala	Thr	Phe	Leu	Lys	Asn	Pro	Phe
			740					745					750		
Gly	Ala	Phe	Thr	Ile	Ile	Leu	Val	Ala	Ile	Ala	Val	Val	Ile	Ile	Ile
		755					760					765			
Tyr	Leu	Ile	Tyr	Thr	Arg	Gln	Arg	Arg	Leu	Cys	Met	Gln	Pro	Leu	Gln
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Asn	Leu	Phe	Pro	Tyr	Leu	Val	Ser	Ala	Asp	Gly	Thr	Thr	Val	Thr	Ser
785					790					795					800
Gly	Asn	Thr	Lys	Asp	Thr	Ser	Leu	Gln	Ala	Pro	Pro	Ser	Tyr	Glu	Glu
				805					810					815	
Ser	Val	Tyr	Asn	Ser	Gly	Arg	Lys	Gly	Pro	Gly	Pro	Pro	Ser	Ser	Asp
			820					825					830		
Ala	Ser	Thr	Ala	Ala	Pro	Pro	Tyr	Thr	Asn	Glu	Gln	Ala	Tyr	Gln	Met
		835					840					845			
Leu	Leu	Ala	Leu	Val	Arg	Leu	Asp	Ala	Glu	Gln	Arg	Ala	Gln	Gln	Asn
	850					855					860				
Gly	Thr	Asp	Ser	Leu	Asp	Gly	Gln	Thr	Gly	Thr	Gln	Asp	Lys	Gly	Gln
865					870					875					880
Lys	Pro	Asn	Leu	Leu	Asp	Arg	Leu	Arg	His	Arg	Lys	Asn	Gly	Tyr	Arg
				885					890					895	
His	Leu	Lys	Asp	Ser	Asp	Glu	Glu	Glu	Asn	Val					
			900					905							

32

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACACGCAAGA GATCTAGACG CGCCTCAT

28

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCGTCCAGAC TCTAGAGGTA GGGC

24

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGACTCCATT CTAGATTAAT GAGTTGCATT

30

33

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAAAGTCGGA GTCTAGAGTC TAGTTCGGAA A

31

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 33 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGATAAGTG GTCTAGATCT AAGCGTAGCT ACG

33

## WHAT IS CLAIMED IS:

1. A non-defective recombinant adenovirus containing a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence, said virus capable of expressing said subunit protein.

2. The adenovirus according to claim 1 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

3. An immunogenic composition comprising a non-defective recombinant adenovirus and a suitable pharmaceutical carrier, wherein said recombinant adenovirus comprises a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence and said virus is capable of expressing said subunit protein *in vivo* in an animal.



4. The composition according to claim 3 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

5. The composition according to claim 3 wherein said protein gene encodes an additional cytomegalovirus subunit protein fragment or a selected cytomegalovirus subunit protein.

6. The composition according to claim 3 wherein said gB subunit fragment is about amino acid 1 to about amino acid 303 of SEQ ID NO:2.

7. The composition according to claim 3 wherein said adenovirus is selected from the group consisting of an adenovirus type 5, adenovirus type 4 and adenovirus type 7 strain.

8. The composition according to claim 7 wherein said gB subunit fragment is obtained from the Towne strain cytomegalovirus, and the adenovirus is type 5.

9. The use of a non-defective recombinant adenovirus comprising a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence and said virus being capable of expressing said subunit protein *in vivo* in an animal, in the preparation of a CMV vaccine.

10. The use according to claim 9 wherein said fragment is selected from the group consisting of:

- (a) the fragment spanning about amino acid 1 to about amino acid 303,
- (b) the fragment spanning about amino acid 1 to about amino acid 700,
- (c) the fragment spanning about amino acid 1 to about amino acid 465,
- (d) fragments spanning about amino acid 155 to about amino acid 303, and
- (e) smaller fragments of (a) through (d) of SEQ ID NO:2.

11. The use according to claim 9 wherein said adenovirus is present in an effective amount of between  $10^5$  to  $10^8$  plaque forming units.

12. An immunogenic composition comprising a gB subunit protein fragment containing at least one CTL epitope expressed in a recombinant adenovirus vector.

13. The composition according to claim 12 wherein said fragment is selected from the group consisting of:

- (a) the fragment spanning about amino acid 1 to about amino acid 303,
- (b) the fragment spanning about amino acid 1 to about amino acid 700,
- (c) the fragment spanning about amino acid 1 to about amino acid 465,
- (d) fragments spanning about amino acid 155 to about amino acid 303, and
- (e) smaller fragments of (a) through (d) of SEQ ID NO:2.

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FIG. IA

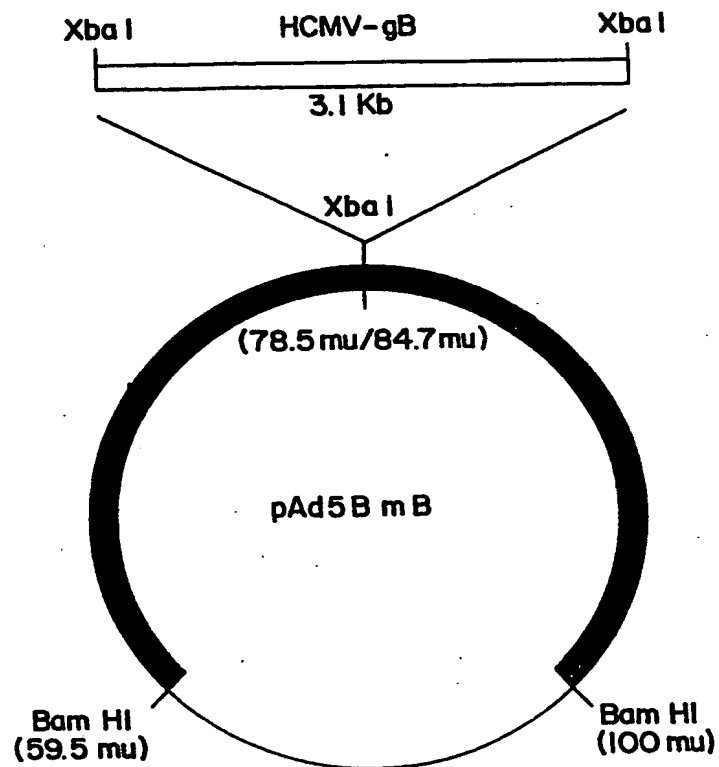
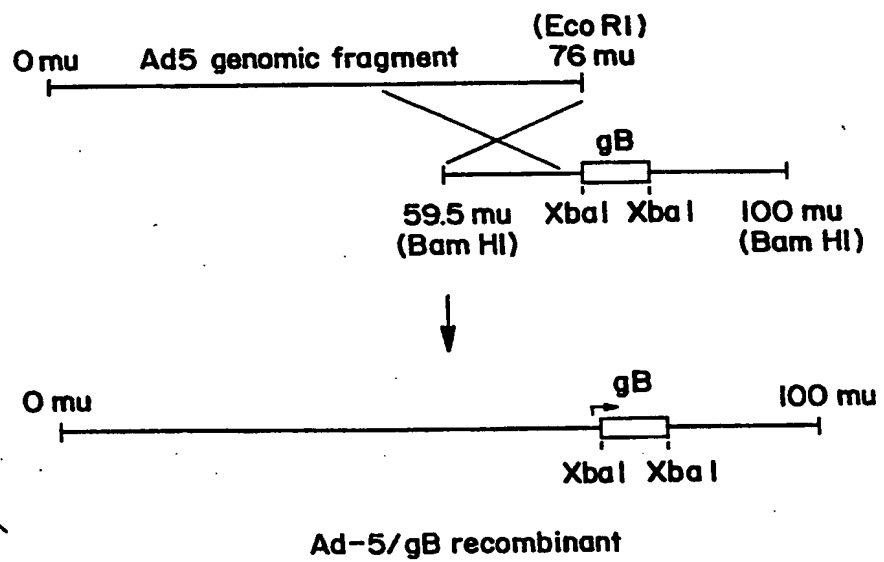


FIG. IB



## INTERNATIONAL SEARCH REPORT

In. ational application No.

PCT/US94/04180

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : Please See Extra Sheet.

US CL : 424/88, 89; 435/235.1, 69.3, 5, 69.1, 172.3; 436/543; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 89; 435/235.1, 69.3, 5, 69.1, 172.3; 436/543; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

INTELLIGENETICS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	THE EMBO JOURNAL, Volume 5, No. 11, issued November 1986, Cranage et al., "Identification Of The Human Cytomegalovirus Glycoprotein B Gene and Induction of Neutralizing Antibodies via Its Expression in Recombinant Vaccinia Virus", pages 3057-3063, see entire document.	1-13
Y	US, A, 4,920,209 (DAVIS ET AL.) 24 April 1990, see entire document.	1-13

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z"

document member of the same patent family

Date of the actual completion of the international search

22 JULY 1994

Date of mailing of the international search report

02 AUG 1994

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**A. CLASSIFICATION OF SUBJECT MATTER:**

IPC (5):

A61K 39/00, 39/12; C12N 7/00, 15/00; C12P 21/06; C12Q 1/70; G01N 33/531; A01N 43/04